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1/23

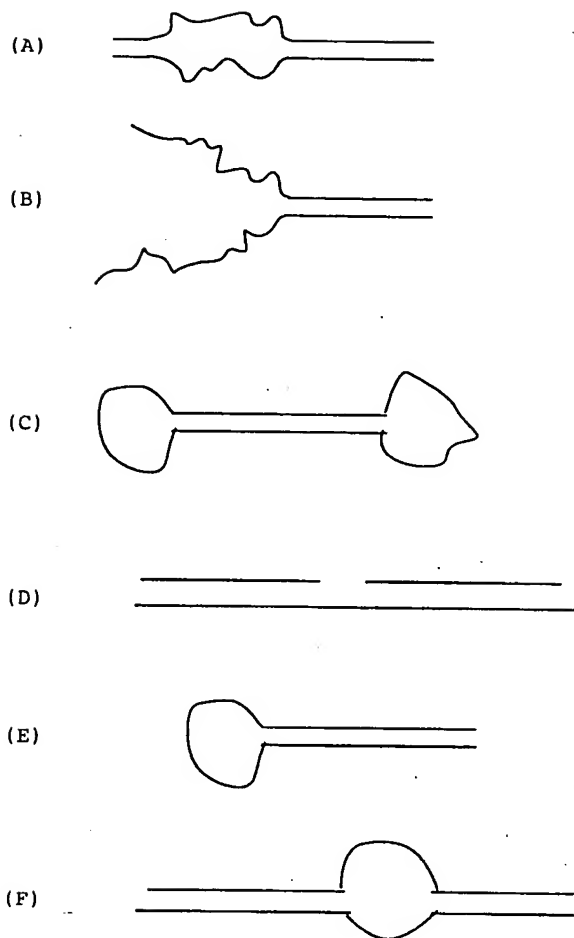


Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

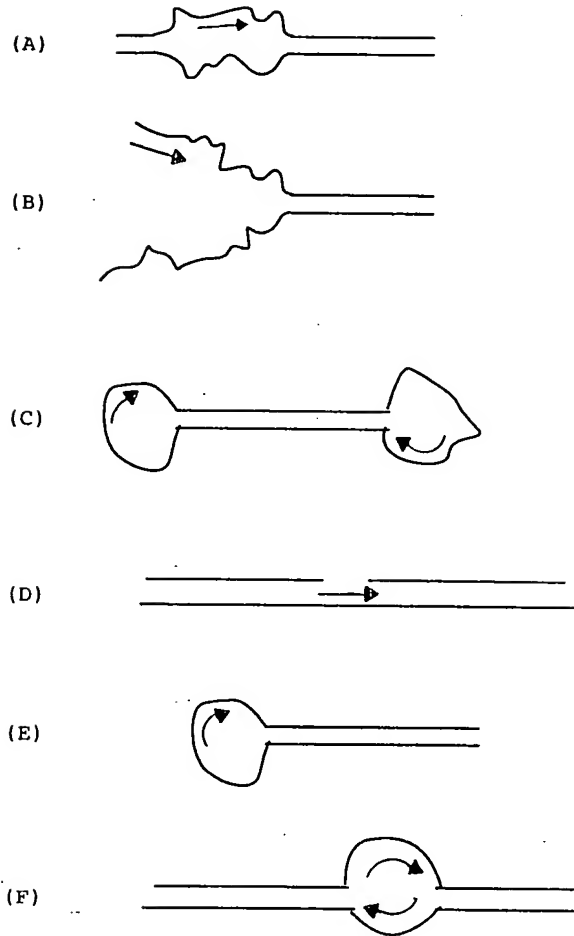


Figure 2 (A-F)

Functional Forms of the Construct

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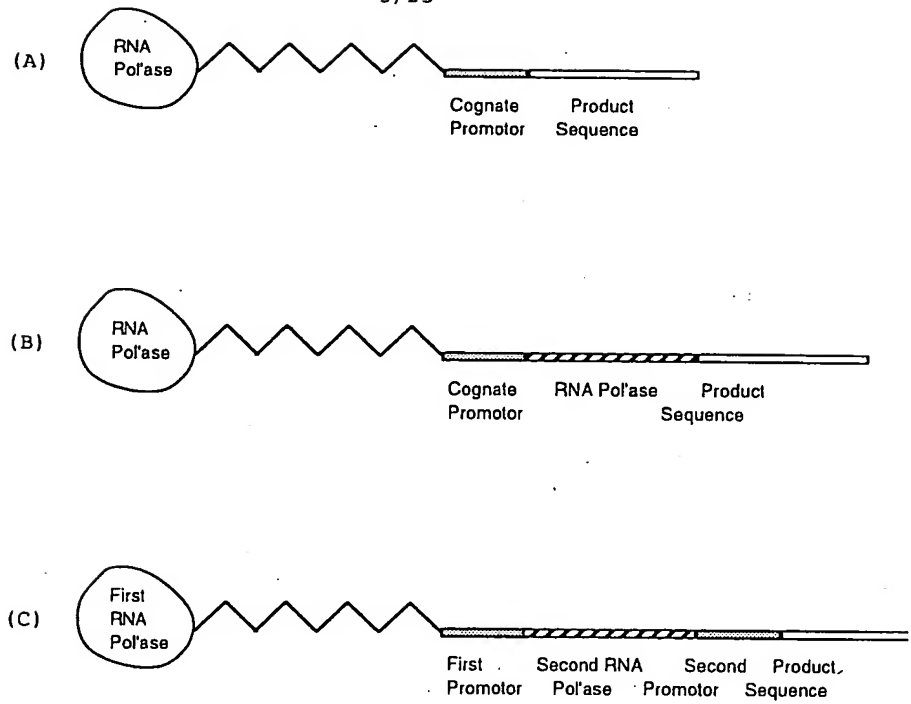


Figure 3 (A-C)

Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette

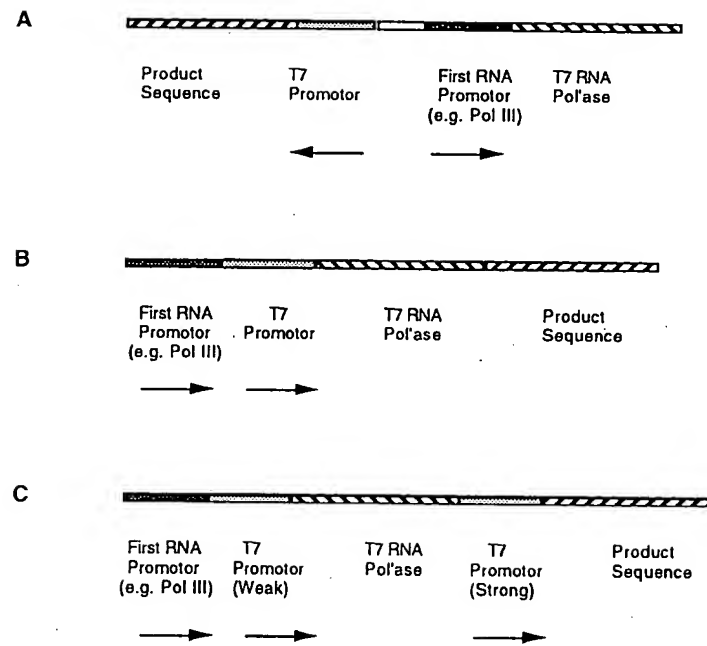


Figure 4 (A-C)

Three Constructs with Promoters for Endogenous RNA Polymerase

M13mp18. Seq Length: 7250

1.	AATGCTACTA	CTATTAGTAG	AATTGATGOC	ACCTTTTCAG	CTOGOGOOOC
51.	AAATGAAAAT	ATAGCTAAAC	AGGTTATTGA	OCATTTGOGA	AATGTATCTA
101.	ATGGTCAAAC	TAAATCTACT	OGTTOGCAGA	ATTGGGAATC	AACTGTTACA
151.	TGGAATGAAA	CTTCAGACA	COGTACTTTA	GTTGCATATT	TAAAACATGT
201	TGAGCTACAG	CACCAGATTC	AGCAATTAAG	CTCTAAGCCA	TOGCAAAAA
251	TGAOCTCTTA	TCAAAAGGAG	CAATTAAGG	TACTCTCTAA	TOCTGAOCTG
301.	TTGGAGTTTG	CTTOGGTCT	GGTTOGCTTT	GAAGCTOGAA	TAAAAAGOG
351.	ATATTTGAAG	TCCTTOGGGC	TTCCTCTTAA	TCTTTTGTAT	GCAATTOGCT
401.	TTGCTTCTGA	CTATAATAGT	CAGGGTAAAG	ACCTGATTTT	TGATTTATGG
451.	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA	TTTGAGGGGG	ATTCAATGAA
501.	TATTTATGAC	GATTCOGCAG	TATTGGAGGC	TATOCAGTCT	AAACATTTTA
551.	CTATTACOOO	CTCTGGCAAA	ACTTCTTTTG	CAAAAGOOCTC	TOGCTATTTT
601.	GGTTTTTATC	GTOGTCTGGT	AAAGAGGGGT	TATGATAGTG	TTGCTCTTAC
651.	TATGOCTOGT	AATTOCTTTT	GGOGTTATGT	ATCTGCATTA	GTTGAATGTG
701.	GTATTOCTAA	ATCTCAACTG	ATGAATCTTT	CTAOCGTGAA	TAATGTTGTT
751.	COGTTAGTTC	GTTTTATTAA	CGTAGATTTT	TCTTDOCAAC	GTOCTGACTG
801.	GTATAATGAG	CCAGTTCTTA	AAATOGCATA	AGGTAATTCA	CAATGATTAA
851.	AGTTGAAATT	AAOCATCTC	AAGOOCAATT	TACTACTOGT	TCTGGTGTTC
901.	TOGTACGGGC	AAGCTTATT	CACTGAATGA	GCAGCTTTGT	TACGTTGATT
951.	TGGGTAATGA	ATATCOGGTT	CTTGTCGAAG	ATTACTCTTG	ATGAAGGTCA
1001	GOCAGOOCTAT	GOGOOCTGTC	TGTACACOGT	TCATCTGTCC	TCTTTCAAAG
1051	TTGGTCAGTT	CGGTTOOCTT	ATGATTGAOC	GTCTGOGOOCT	CGTTTOGGCT
1101	AAGTAACATG	GAGCAGGTGG	CGGATTTTGA	CACAATTTAT	CAGGOGATGA
1151	TACAAATCTC	CGTTGTACCTT	TGTTTGGOGC	TTGGTATAAT	OGCTGGGGGT
1201	CAAAGATGAG	TGTTTTAGTG	TATTCCTTGG	OCTCTTTOGT	TTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCTTGTGTA	GTGGCATTAC	GTATTTTACC	CGTTTAATGG	AAACTTCTCTC
1301	ATGAAAAAGT	CTTTAGTCTCT	CAAAGCCTCT	GTAGGCGTTG	CTAAGCTCTGT
1351	TCGATGCTG	TCTTTGCTG	CTGAGGGTGA	CGATCCCGCA	AAAGGGGCT
1401	TTAACTCCCT	GCAAGCTCA	GCGAAGCAAT	ATATCGGTGA	TGGTGGGGG
1451	ATGGTTGTTG	TCATTGTCTG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCACTCTG	AAAGCAAGCT	GATAAAGCGA	TACAATTAAA	GGCTCTTTT
1551	GGAGCTTTT	TTTTTGAGA	TTTCAACGT	GAAAAAATTA	TTATTCGCA
1601	TTCTTTAGT	TGTTCTTTC	TATTCTCACT	CGCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AAGCCATAC	AGAAATTCA	TTTACTAAGC	TCTGGAAAGA
1701	CGACAAACT	TTAGATGTT	AGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGCT	TGTAGTTGT	ACTGGTGAAG	AAACTCAGTG	TTACGGTACA
1801	TGGGTTCTA	TTGGGCTTG	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGGGT	TCTGAGGGT	GCGGTTCTGA	GGGTGGGGT	ACTAAAGCTC
1901	CTGAGTAAG	TGATACAAG	ATTCGGGGCT	ATACTTATAT	CAAGCTCTC
1951	GAGGGCACTT	ATCGGCTGG	TACTGAGCAA	AAGCGCTA	ATCTAATCC
2001	TTCTCTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTCCGAAA	TAGGCAGGG	GCATTAACGT	TTTATAGGC	CACTGTTACT
2101	CAAGGCACTG	AAGCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TATGAAGCTT	ACTGGAAGG	TAAATTCAGA	GACTGCGCTT
2201	CAAGGCACTG	AAGCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TGCTCAAGC	TCCTGTCAAT	GCTGGGGGG	GCTCTGGTGG
2201	TCATTCTGG	CTTTAATCAA	GATCATTGG	TTTGTGAATA	TCAAGGCCAA
2251	TGTTCTGAG	TGCTCAAGC	TCCTGTCAAT	GCTGGGGGG	GCTCTGGTGG
2301	TGGTTCTGGT	GGGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGGGTTCTG
2351	AGGGTGGGG	CTCTGAGGGA	GGGGTTCCG	GTTGGTGGCTC	TGGTTCCGGT
2401	GATTTTGATT	ATGAAAAGAT	GGCAAGCGCT	AATAAGGGGG	CTATGAAGCA
2451	AAATGCCGAT	GAAAAGCGC	TACAGTCTGA	CGCTAAAGGC	AACTTGATT

Figure 5

M13mp18 Nucleic Acid Sequence

```

2501 CTGTGCTAC TGATTAAGGT GCTGCTATCG ATGGTTTCAT TGGTGAOGTT
2551 TOGGGOCCTG CTAATGGTAA TGGTGCTACT GGTGATTTTG CTGGCTCTAA
2601 TTOCCAAATG GCTCAAGTOG GTGAOGGTGA TAATTCAOCT TTAATGAATA
2651 ATTTGCGTCA ATATTTACCT TOOCTOOCCTC AATOGGTTGA ATGTGGOOCT
2701 TTTGTCTTTA GCGCTGGTAA AOCATATGAA TTTTCTATTG ATTGTGACAA
2751 AATAAACTTA TTOGTGGTG TCTTTGOGTT TCTTTTATAT GTTGOCACCT
2801 TTATGTATGT ATTTTCTACG TTTGCTAACA TACTGOGTAA TAAGGAGTCT
2851 TTATCATGOC AGTTCTTTTG GGTATTOOCT TATTATTGOG TTTOCTOGGT
2901 TTOCTTCTGG TAACTTTGTT OGGCTATCTG CTTACTTTTC TAAAAAGGG
2951 CTTOGGTAAG ATAGCTATTG CTATTTCACT GTTCTTGCT CTTATTATTG
3001 GGCTTAACTC AATCTTGTG GGTATCTCT CTGATATTAG CGCTCAATTA
3051 OOCCTGACT TTGTCAGGG TGTTCAGTTA ATTCTOOGT CTAATGOGCT
3101 TOOCTGTTTT TATGTTATTC TCTCTGTAAG GGCTGCTATT TTCATTTTTG
3151 ACGTTAAACA AAAAATOGTT TCTTATTTGG ATTGGGATAA ATAATATGGC
3201 TGTTTATTTT GTAACGGCA AATTAGGCTC TGGAAAGACG CTGTTAGOG
3251 TTGGTAAGAT TCAGGATAAA ATTGTAGCTG GGTGCAAAAT AGCAACTAAT
3301 CTTGATTTAA GGCTTCAAAA OCTOOOGCAA GTGGGAGGT TOGCTAAAAAC
3351 GOCTOGOGTT CTTAGAATAC CGGATAAGOC TTCTATATCT GATTTGCTTG
3401 CTATTGGGOG CGGTAATGAT TOCTAOGAATG AAAATAAAAA CGGCTTGCTT
3451 GTTCTOGATG AGTGOGGTAC TTGGTTTAAT AOOOGTTCTT GGAATGATAA
3501 GGAAAGACAG OCGATTATTG ATTGGTTTCT ACTGCTOCT AAATTAGGAT
3551 GGGATATTAT TTTTCTTGTT CAGGACTTAT CTATTGTTGA TAAACAGGOG
3601 CGTTCTGCAT TAGCTGAACA TGTTGTTTAT TGTOGTGCTC TGGACAGAAT
3651 TACTTTACCT- TTTGTGGTA CTTTATATTC TCTTATTACT GGCTOGAAAA
3701 TGCTCTGOC TAAATTACAT GTTGGOGTTG TTAATATGG CGATTCTCAA
3751 TTAAGCOCTA CTGTTGAGOG TTGGCTTTAT ACTGGTAAGA ATTTGTATAA
3801 OGCATATGAT ACTAAACAGG CTTTTTCTAG TAATTATGAT TOGGTGTTT

```

Figure 5

M13mp18 Nucleic Acid Sequenc

3851	ATTCTTATTT	AACGCTTAT	TTATCACAAG	GTCGGTATTT	CAAACCATTA
3901	AATTTAGGTC	AGAAGATGAA	ATTAACATAA	ATAATATTGA	AAAAGTTTTC
3951	TOGCGTTCTT	TGCTTGCGA	TTGGATTGCG	ATCAGCATTT	ACATATAGTT
4001	ATATAAOCOA	AOCTAAGCOG	GAGGTAAAA	AGGTAGTCTC	TCAGACCTAT
4051	GATTTTGATA	AATTCACAT	TGACTCTTCT	CAGCGTCTTA	ATCTAAGCTA
4101	TCGCTATGTT	TTCAAGGATT	CTAAGGGAAA	ATTAATTAAT	AGCGAOGATT
4151	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTTATG	TACTGTTTCC
4201	ATTAAAAAAG	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTTGTTT
4251	TCTTGATGTT	TGTTTCATCA	TCTTCTTTTG	CTCAGGTAAT	TGAAATGAAT
4301	AATTGCGCTC	TGCGCGATTT	TGTAACCTGG	TATTCAAAGC	AATCAGGOGA
4351	AATCGGTTATT	GTTTCTCCCG	ATGTAAAAGG	TACTGTTACT	GTATATTCAT
4401	CTGACGTTAA	AOCTGAAAAT	CTACGCAATT	TCTTTATTTT	TGTTTAAOGT
4451	GCTAATAATT	TTGATAATGGT	TGGTTCAATT	OCTTCCATAA	TTTCAAGATA
4501	TAATCCAAAC	AATCAGGATT	ATATTGATGA	ATTGOCATCA	TCTGATAATC
4551	AGGAATATGA	TGATAATTCC	GCTCCTTCTG	GTGGTTTCTT	TGTTCCGCAA
4601	AATGATAATG	TTACTCAAAC	TTTTAAAATT	AATAACGTTT	GGGCAAGGA
4651	TTTAATAOCA	GTTGTGGAAT	TGTTTGTAAG	GTCTAATACT	TCTAAATCCT
4701	CAATGTATT	ATCTATTGAC	GGCTCTAATC	TATTAGTTGT	TAGTGCTCCT
4751	AAAGATATTT	TAGATAAOCCT	TOCTCAATTC	CTTTCTACTG	TTGATTTGCC
4801	AACTGAOCAG	ATATTGATTG	AGGGTTTGAT	ATTTGAGGTT	CAGCAAGGTG
4851	ATGCTTTAGA	TTTTTCATTT	GCTGCTGGCT	CTCAGCGTGG	CACGTTTGCA
4901	GGCGGTGTTA	ATACTGAOCG	OCTCAOCTCT	GTTTATATCT	CTGCTGGTGG
4951	TTGGTTGGGT	ATTTTAAATG	GCGATGTTTT	AGGGCTATCA	GTTGCGGCAT
5001	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCCAAG	TATTCTTAAG
5051	CTTTCAGGTC	AGAAGGGTTC	TATCTCTGTT	GGCCAGAATG	TCCCTTTTAT
5101	TAAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCCAAG	TATTCTTAAG
5151	CGATTGAGCG	TCAAAATGTA	GGTATTTCCA	TGAGCGTTTT	TCTGTTTGCA

Figure 5

M13mp18 Nucleic Acid Sequence

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5201 ATGGCTGGCG GTAATATTGT TCTGGATATT AOCAGCAAGG COGATAGTTT
5251 GAGTTCTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA AGAAGTATTG
5301 CTACAAOOGT TAATTTGOGT GATGACAGA CTCTTTTACT OGGTGGOCTC
5351 ACTGATTATA AAAACACTTC TCAAGATTCT GGOGTACOGT TOCTGTCTAA
5401 AATOCCTTTA ATOGGOCTOC TGTTTAGCTC OOGCTCTGAT TOCAAOGAGG
5451 AAAGCAOGTT ATAOGTGCTC GTCAAAGCAA OCATAGTAOG CGOOGTGTAG
5501 OGGOGCATT AOGOGGOGG GTGTGGTGGT TAGOGGAGC GTGAOOGCTA
5551 CACTTGOCAG OGOCTAGOG OOGGCTOCTT TCGCTTTCTT COCTTOCTTT
5601 CTGOCAGT TOGOGGCTT TDOOGTCAA GCTCTAAATC GGGGGCTOOC
5651 TTTAGGGTTC OGATTTAGTG CTTTAOGGCA OCTOGAOOOC AAAAACTTG
5701 ATTTGGGTGA TGGTTCAOGT AGTGGGOCAT OGOOGTGATA GACGGTTTTT
5751 OGOOCTTTGA OGTTGAGTC CAOGTTCTTT AATAGTGGAC TCTTGTCCA
5801 AACTGGAACA AACTCAAOC CTATCTOGG CTATTCTTTT GATTTATAAG
5851 GGATTTTGOC GATTTGGAA OCAOCATCAA ACAGGATTTT OGOCTGCTGG
5901 GGCAAOCAG OGTEGAOOG TTGCTGCAAC TCTCTCAGG OCAGGOGGTG
5951 AAGGGCAATC AGCTGTTGOC OGTCCTGCTG GTGAAAAGAA AAACCAOCT
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6101 OGCAATTAAT GTGAGTTAGC TCACTCATTA GGCAOOOCAG GCTTTACT
6151 TTATGCTTCC GGCTOGTATG TTGTGTGGAA TTGTGAGOGG ATAACAATTT
6201 CACACAGGAA ACAGCTATGA OCATGATTAC GAATTOGAGC TOGGTACOOG
6251 GOGATCTCT AGAGTOGAOC TGCAGGCATG CAAGCTTGGC ACTGGOOGTG
6301 GTTTTACAAC GTGTGACTG GGAAAAOCT GOGTTAOC AACTTAATOG
6351 OCTTGACGA CAATCOOCTT TOGOCAGCTG GOGTAATAGC GAAGAGGOOC
6401 GCACOGATOG COCTTOCAA CAGTTGOGCA GOGTGAATGG OGAATGGOGC
6451 TTTGCTGGT TTOGGCACC AGAAGOGGTG OOGGAAAGCT GGCTGGAGTG
6501 OGATCTOCT GAGGOOGATA OGTGTGTGT COOCTCAAAC TGGCAGATGC

```

Figure 5

M13mp18 Nucleic Acid Sequence

6551	ACGGTTAOGA	TGOGGOCATC	TACAOCACOG	TAAOCTATCC	CATTACGGTC
6601	AATCOGGOGT	TTGTTCCAC	GGAGAATCOG	ACGGGTTGTT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGOCAGACG	CGAATTATTT
6701	TTGATGGOGT	TOCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAAATTA
6751	ACGCGAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTCC	TGTTTTTGGG	GCTTTTCTGA	TTATCAACOG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACOGTT	CATCGATTCT	CTTGTTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCCT	TTGTAGATCT	CTCAAAAATA
6951	GCTAOCCTCT	COGGCATGAA	TTTATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGCCTTTC	TCACOCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GCGTTGAAAT	AAAGGCTTCT	COOGCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TTTATGCTCT	GAGGCTTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M ₁₃			
POSITION	5' . . . 3'	POSITION	
645	AGCAACACTATCATA	631	M ₁₃ /1
615	ACGAACGATAAAAAAC	601	M ₁₃ /2
585	TTTTGCAAAAGAAGT	571	M ₁₃ /3
555	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGCGGAATG	511	M ₁₃ /5
495	TGAATCCCCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTTACCGTG	421	M ₁₃ /8
405	AGGAAAGCGGATTGC	391	M ₁₃ /9
375	AGGAAGCCCGAAAGA	361	M ₁₃ /10
COMPLEMENTARY TO SS PHAGE DNA			
POSITION	5' . . . 3'	POSITION	
351	ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCITTTTGATGCAAT	386	M ₁₃ /12
391	CTATAACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTTGA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATCCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAACTTC	506	M ₁₃ /18
511	TCGCTATTTTGGTTT	526	M ₁₃ /19
531	AAACGAGGGTTATGA	546	M ₁₃ /20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

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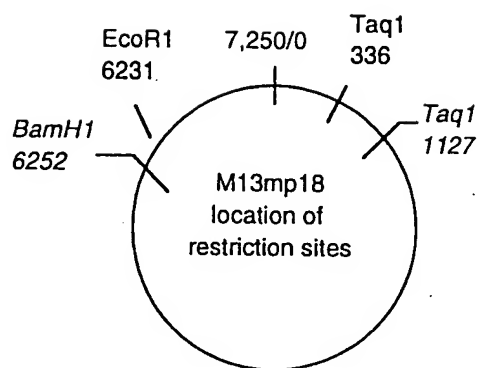
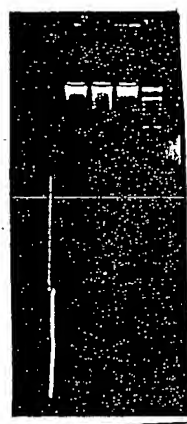


Figure 7

Appropriate M13mp18 Restriction Sites

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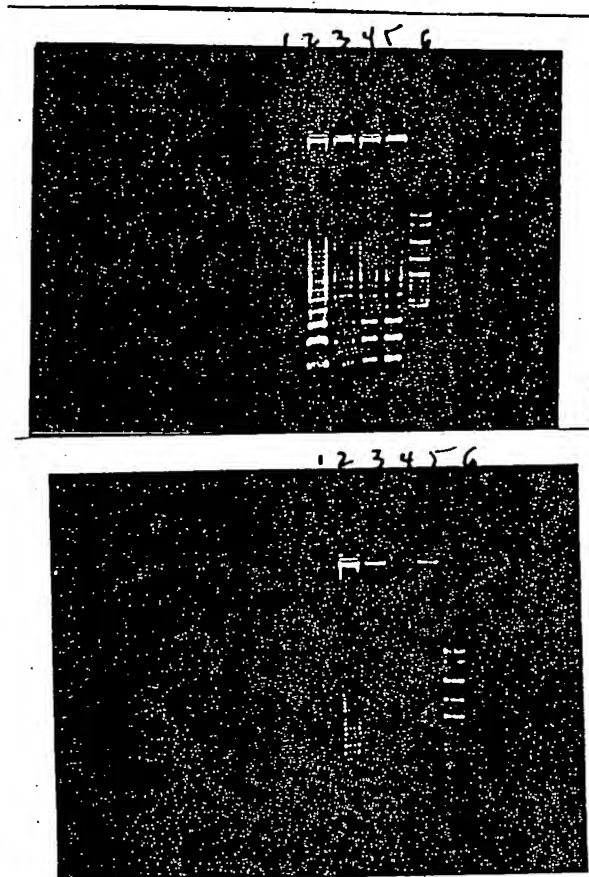
Lane 1: from calf thymus + Taq digested mp18 amplification reaction
Lane 2: from Taq digested mp18 amplification reaction
Lane 3: from calf thymus amplification reaction
Lane 4: øX174 Hinf1 size marker

Figure 8



Lane 1: no template
Lane 2: mp18 template, phosphate buffer
Lane 3: MspI/pBR322 size marker
Lane 4: mp18 template, MOPS buffer

Figure 9



Top= (+) Template

Bottom= (-) Template

Lane 1: phosphate buffer

Lane 2: MES

Lane 3: MOPS

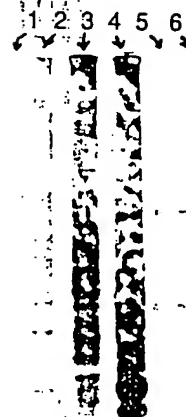
Lane 4: DMAB

Lane 5: DMG

Lane 6: pBR322/Mspl size marker

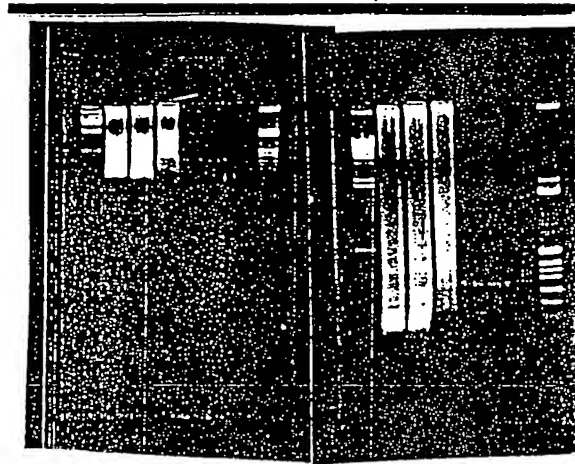
Figure 10

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Lane 1: DMAB buffer, no template
Lane 2: DMAB buffer, mp18 template
Lane 3: DMG buffer, no template
Lane 4: DMG buffer, mp18 template
Lane 5: No reaction
Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: phiX174/Hinf1 marker

Figure 12

18/23

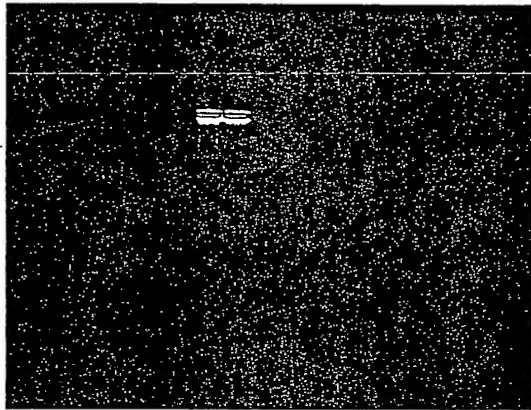
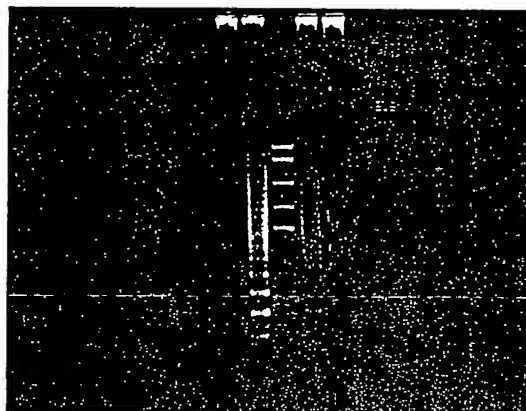


Figure 13

19/23

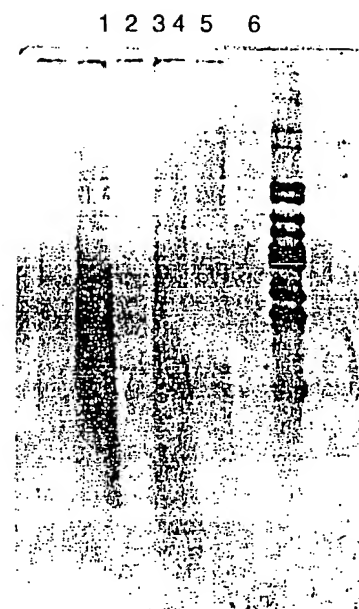
1 2 3 4 5 6



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 14

20/23



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 15

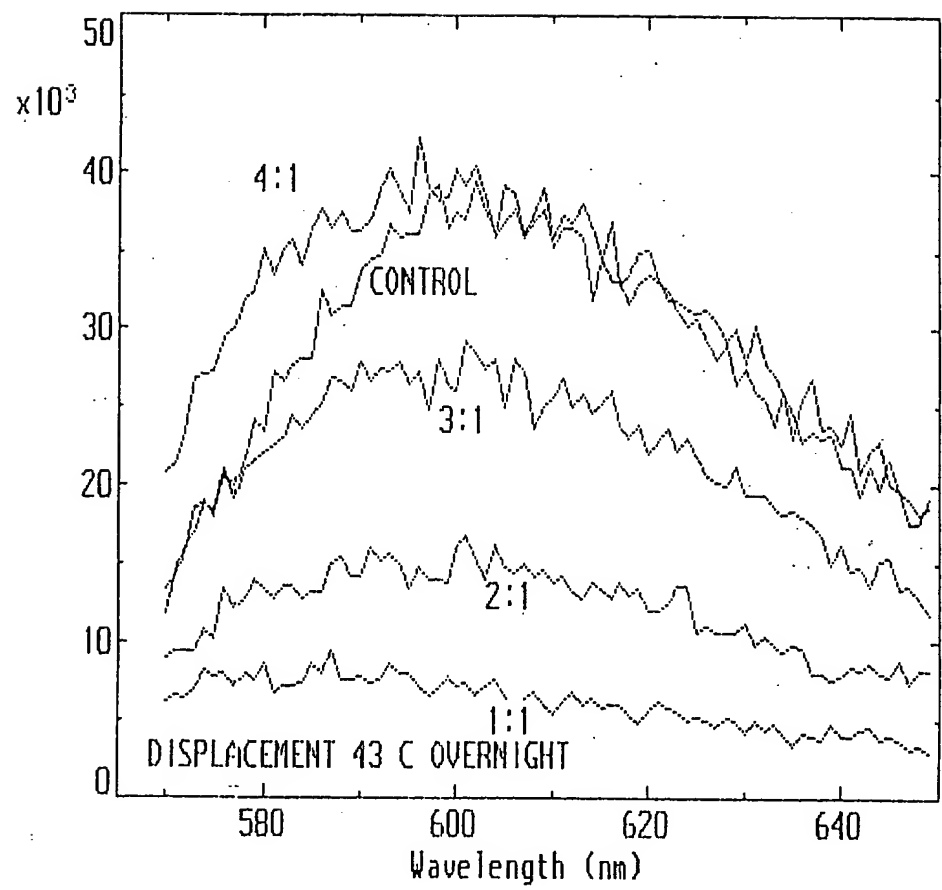


Figure 16

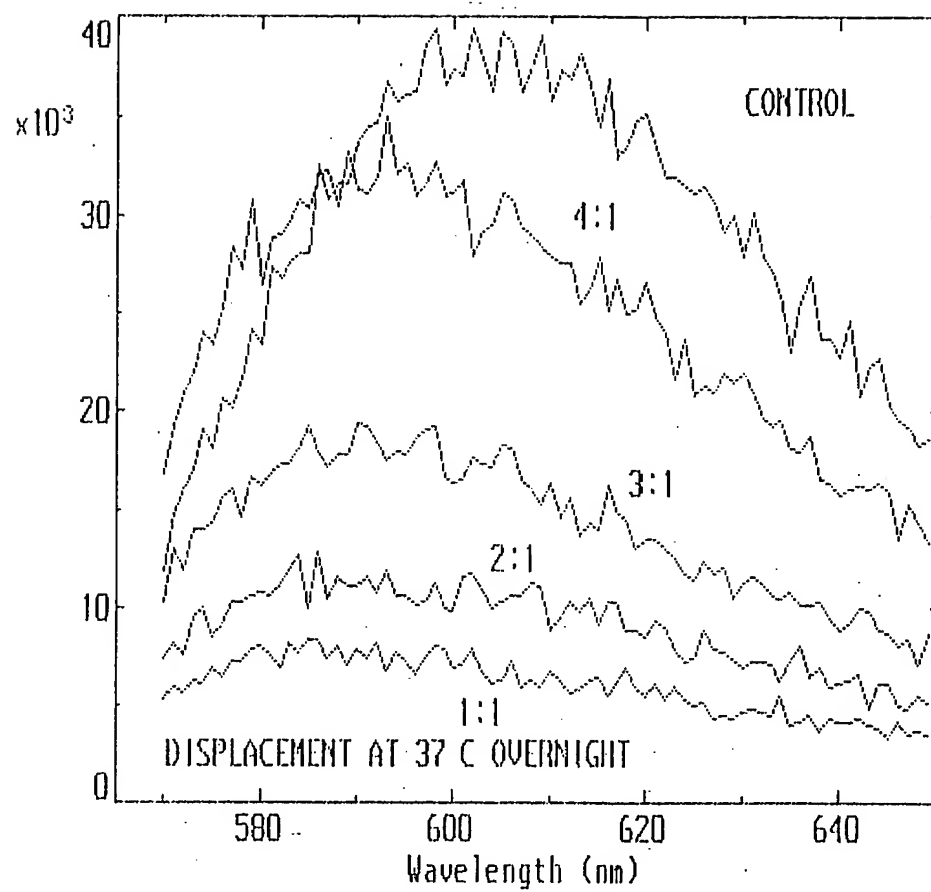


Figure 17

23/23

pIBI 31-BH5-2

fmet AUG of Lac z (T7 Promotor region....
 LAC PROMOTOR.ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
 oligo 50-mer 3'- tac t*aa t'gc ggt* ct*a t'ag t'Vt aat* tat* gct* gag t'ga t'al* c-5'
 10 base insert

T7 RNA Start {«« T3 Promotor Region }
IGGG CTC ICCT TTA GTG ACG GTT AAT
...»»} «- T3 Start Signal

pIBI 31 BSII/HCV

fmet AUG of Lac z (T3 Promotor region ->) T3 RNA Start
 LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
 oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggi* t'V--10 base insert--.....

(← T7 Promotor Region)
 MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
 ← T7 Start Signal
 5'-ct'a t'ag t'ga gt'c gt'a tt'a at'.....

Figure 18